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Research Article

Influence of Glucosaminilemuramylpentapeptide on the Functional Peculiarities of the Respiratory System and the Dynamics of IL-6 in Patients with Infiltrative Tuberculosis Combined with Chronic Bronchitis

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Abstract
The aim of our study was to evaluate the functional peculiarities of the respiratory system and the dynamics of IL-6 in patients with infiltrative tuberculosis combined with chronic bronchitis.
Materials and methods. There were examined 40 patients with infiltrative tuberculosis combined with chronic bronchitis: 18 patients receiving basic therapy according to the category and 22 patients who were added glucosaminilemuramylpentapeptide to basic therapy. Determination of IL-6 in bronchoalveolar content was performed by ELISA analysis.
Results. The additional inclusion of glucosaminilemuramylpentapeptide into the complex therapy of patients with infiltrative tuberculosis combined with chronic bronchitis in acute phase manifests itself with positively aimed dynamic changes of the bronchial mucous membrane according to the data of endoscopy. The standard scheme of treatment combined with immune modulator affects the recovery of lung function in comorbidity of pathologies explaining it as the action of the means aimed at enhancing of sanogenetic mechanisms of liquidation of combination of specific and nonspecific inflammation in the broncho-pulmonary system, and thus – the reduction of the basis for secondary bronchial obstruction. The results of this study indicate the advisability of administration of immune modulator of muramylpeptide series.

Keywords
infiltrative tuberculosis; chronic bronchitis; spirometry; fibrobronchoscopy; bronchoalveolar content; IL-6

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Problem statement and analysis of the recent research

According to the literature data, about 10% of people already infected with Mycobacterium tuberculosis, can contract tuberculosis [1, 3, 9, 10]. Vital activity of Mycobacterium Tuberculosis, in other cases, is controlled by the immune system and this pathogen can be in alveolar macrophages or in the lymph nodes [4-6, 8]. According to the data of leading scientists, in this category of population, due to weakening of the immune system, the disease develops in the reactivation of latent tuberculosis infection [1, 4, 9].

According to medical literature chronic bronchitis is at one of the first places among prevalence in patients with respiratory diseases, namely among chronic nonspecific diseases. According to the statistics, the figure of the above-mentioned morbidity has decreased only at 6%, and this is because of the low level of complaints to doctors of such patients.

As a result of deformation and violation of bronchial trophism, drainage deterioration of their function, which is a favorable basis for the breeding of pathogens and fibrotic changes in the lungs, chronic nonspecific diseases of respiratory organs develop. In combination of pathologies due to inflammatory cell activation, hypoxia of lung tissue is observed. Feshchenko Yu.I. (2012) described that these violations lead to the so-called oxide stress and changes in levels of inflammatory mediators. According to some native authors, acute phase proteins manifest the greatest expression of inflammation among biomarkers [2].

Leading scientists' works have proved that the development and course of tuberculosis depends on the state of the immune system [2, 4-7, 9]. Cytomorphological and functional immunity disorders, leading to slower processes of regression of pathological changes in the lung tissue occur in patients with pulmonary tuberculosis [5, 6, 11]. Tissue hypoxia that occurs in patients with tuberculosis, leads to fibrous sclerotic changes in the lungs [11].

Objective: To determine the effect of glucosaminilemuramylpentapeptide on the state of bronchial mucous membrane, external respiratory function and the level of IL-6 in

bronchoalveolar content in patients with infiltrative tuberculosis combined with chronic bronchitis.

1. Materials and methods

The study of the efficiency of the use of immune modulator glucosaminilemuramylpentapeptide (Liasten, "Enzym" Vinnytsia, Ukraine) in patients with infiltrative tuberculosis combined with chronic bronchitis was performed. Verification of the diagnosis of infiltrative tuberculosis and its formulation was carried out according to the materials of the Order of MOH Ukraine # 620 from 04.09.2014 – On approval of unified clinical protocols of primary, secondary (specialized) and tertiary (highly specialized) medical care "Tuberculosis". The diagnosis of chronic bronchitis was made according to the International Statistical Classification of Diseases and Related Health Problems, clinical and physical-instrumental signs (productive cough for at least 3 months a year for 2 years or more, the presence of the vesicular breathing with dry rale in the acute phase, in medical history 2-3 exacerbations per year over the past 2 years, roentgenological signs of the walls thickening of the bronchi and the phenomena of pneumosclerosis). There were examined 40 patients with infiltrative tuberculosis combined with chronic bronchitis: group I (comparison) – 18 patients receiving basic therapy according to category (according to the Order of MOH Ukraine #620 from 09/04/2014); group II (basic) – 22 patients who were added glucosaminilemuramylpentapeptide to basic treatment. Standard therapy of pulmonary tuberculosis consisted of isoniaside, rifampicine, ethambutol and pyrazinamide, the doses of which were calculated according to the weight of the patient. Patients with chronic bronchitis were administered anti-inflammatory, mucolytic and bronchodilator therapy. The control group consisted of 15 practically healthy individuals (PHI) without signs of pulmonary diseases and other diseases of internal organs.

The first course of treatment – glucosaminilemuramylpentapeptide was received intramuscularly at a dose of 2 mg, 5 injections with an interval of 5 days between them; in 3 months – the second course lasting 20 days, 1 tablet 2 mg 2 times a day.

All patients in the process of study, a number of pathogenic features of the combination of infiltrative tuberculosis and chronic bronchitis spirometry using spirograph "Spirokom" was performed, and some patients (27 patients), by agreement, were performed FBS using fibrobronchoscope of the company "Olympus BF-20" with simultaneous taking of bronchoalveolar content and evaluation of inflammatory changes of the tracheobronchial tree.

Determination of IL-6 in bronchoalveolar content (BAC) was performed using reagents "Interleukin-ELISA-Best".

These parameters were evaluated using the method of enzyme immunoassay in analyzer "StatFax 303 Plus". To determine the content of interleukins the micro-ELISA secretion assay method with the use of horseradish peroxidase as an indicator enzyme was performed. One type of antibodies was immobilized at the inner surface of plates' pits for microtitering. The second type of monoclonal antibodies to epitope of the studied interleukin molecules was in complexes as conjugate with biotin. Indicating component was horseradish peroxidase conjugate with streptavidin, which has very high affinity to biotin. After incubation and washing peroxidase conjugate with streptavidin was introduced into the pits for further incubation, and then – washing, introduction of substrate and measuring of bound peroxidase. Titration of bronchoalveolar content was performed according to the amount of total protein.

2. Results

In order to assess the effectiveness of glucosaminilemuramylpentapeptide and standard therapy we have analyzed data of FBS in 11 patients of group I and in 16 patients of group II.

Analysis of the data determined on the 30th day of the performed standard treatment in patients of group I that a pronounced catarrhal inflammation has decreased compared with the index before treatment and amounted to 63.64% ($p<0.05$), mild catarrhal inflammation was observed in 18.18% ($p<0.05$). At the 150th day (9 patients) the phenomena of the pronounced catarrhal inflammation decreased 2.9 times, and respectively, mild catarrhal inflammation was observed only in 55.56% of patients ($p<0.05$). Identification signs of bronchial mucosal atrophy were without dynamic changes.

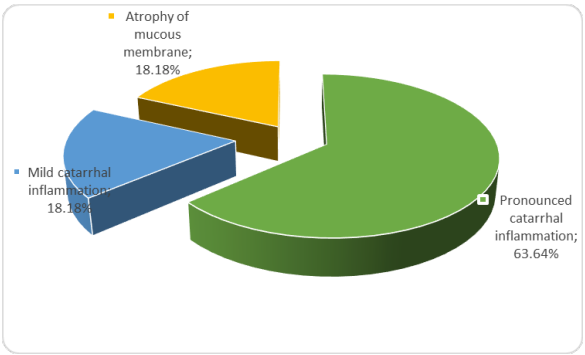

Figure 1. State of mucous membrane of bronchi at the 30th day of treatment of group I

Inclusion of glucosaminilemuramylpentapeptide into the standard treatment regimen of infiltrative tuberculosis combined with chronic bronchitis in acute phase allowed determine the phenomena of the pronounced catarrhal inflammation on the 30th day of treatment in 56.25%. On the 150th day of the therapy (12 patients) there was determined a further reduction ($p<0.05$) of a part of the pronounced catarrhal form of inflammation which was observed in only 16.67% of patients and mild catarrhal inflammation was identified in 41.67% of patients. Complete recovery of the structure of the bronchial mucosa was observed in 33.33% of patients. No significant changes of the identification of bronchial mucosal atrophy in the treatment process of the studied in group II

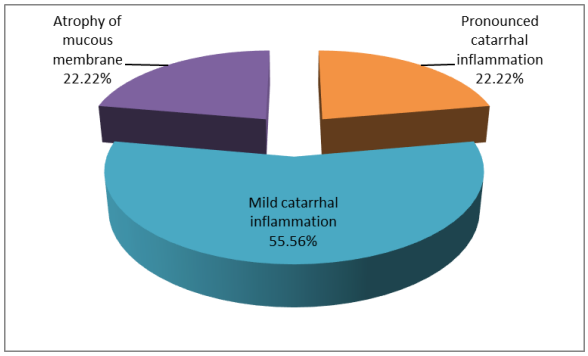

Figure 2. State of mucous membrane of bronchi at the 150th day of treatment of group I

were not determined.

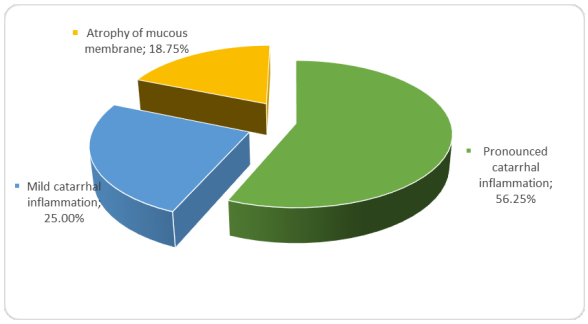

Figure 3. State of mucous membrane of bronchi at the 30th day of treatment of group II

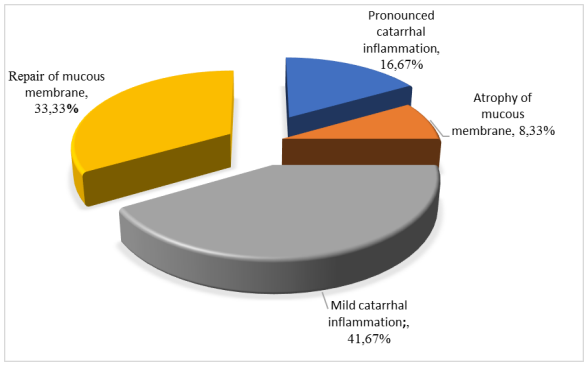

Figure 4. State of mucous membrane of bronchi at the 150th day of treatment of group II

Thus, the additional inclusion of glucosaminilemuramylpentapeptide into the complex therapy of patients with infiltrative tuberculosis combined with chronic bronchitis in acute phase manifests itself with positively aimed dynamic changes of the bronchial mucous membrane according to the data of endoscopy, moreover, the best result was observed in patients who in the complex treatment took two courses: 16 patients – intramuscularly at a dose of 2 mg, 5 injections with an interval of 5 days; in 3 months (12 patients) – the second course lasting 20 days, 1 tablet at a dose of 2 mg 2 times a day of this immune modulator.

In assessment of spirographic indexes in patients of group I, a slight decrease of the main rate indicators of expiratory function (FEV_1 and FVC) was revealed, which makes it impossible to identify the presence of ventilatory failure in this group of patients. However, the stricter changes of MEF_{25}, MEF_{50} and MEF_{75} were observed.

Thus, MEF_{25} was (71.45±2.91) %, which was at 17.46% lower than the control group data ($p1<0.05$), MEF_{50} fell to (73.04±2.23) %, and was lower than 17.01% ($p1<0.05$), compared with data of PHI. There was a similar tendency in the decline of MEF_{75}, which was (66.38±2.16) %, that was at 23.52% lower than data of the control group ($p1<0.05$).

Assessing indexes of spirogram in group I after 30 days of treatment, we have not noted marked changes. However, MEF_{25} was (73.18±2.87) %, which was 1.02 times higher than before the treatment, MEF_{50} at 1.05 times and MEF_{75} at 1.06 times exceeded the corresponding figure. During the 150^{th} day a slight increase of indexes was determined, namely MEF_{25} was (76.6±2.4) %, which at 5.15% increased as for the given rate before the treatment ($p2<0.05$), but 1.21 times less compared to the PHI ($p1<0.05$). MEF_{50} increased to (80.59±2.1) %, and was at 7.55% higher compared with the data before treatment ($p2<0.05$), but compared with the control group this figure was at 9.91% lower ($p1< 0.05$). The growth of index of MEF_{75} (76.84±2.03) %, was at 10.46% higher than data before treatment ($p2<0.05$), but 13.06% lower than in the group of PHI ($p1<0.05$).

The positive effect we have got in patients of group II, who were used immunomodulator glucosaminilemuramylpentapeptide in the complex treatment; that allowed us to note a slight increase of indexes during the 30^{th} day of the treatment: MEF_{25} was (75.33±2.43) %, MEF_{50} - (78.61±1.82) %, and MEF_{75} - (73.06±2.10) %. During the 150^{th} day we've determined further significant growth rates, namely MEF_{25} was (89.12±1.83) %, MEF_{50} - (88.58±1.55) %, and MEF_{75} - (82.46±2.19) %.

Thus, the full course of the intake of glucosaminilemuramylpentapeptide on a background of standard treatment regimen led to the rise of the function of expiratory respiration (FER). In patients of group II the index of MEF_{25} on the 150^{th} day of treatment was 1.2 times, MEF_{50} – 1.1 times and MEF_{75} - 1.1 times better than during the corresponding period in the subgroup III ($p3<0.05$) (Table 2).

However, these options are less than the values in the control group. After analyzing the data of spirography in the second cohort, we have noted changes during the 150^{th} day of the treatment: the MEF_{25} in 1.05 times ($p1<0.05$), MEF_{50} - in 1.02 times ($p1<0.05$), and MEF_{75} - in 1.09 times ($p1<0.05$) less compared with the indexes of PHI.

Thus, the standard scheme of treatment combined with glucosaminilemuramylpentapeptide affects the recovery of lung function in comorbidity of pathologies explaining it as the action of the means aimed at enhancing of sanogenetic

Table 1. The indicators of excretory lung function during treatment in patients with infiltrative tuberculosis depending on the presence of chronic bronchitis, (M±m)

Indexes of spirometry	Groups of the studied persons				p1	p2
	Practically healthy individuals (PHI), n=15	group I, n=18				
		Before treatment	30th day	150th day		
Forced Volume Vital Capacity (FVC) %	101.2±3.25	90.62±0.73	90.59±0.60	92.36±0.42	<0.05	<0.05
Forced Expiratory Volume (FEV_1), %	97.3±1.47	81.56±1.01	84.99±1.27	86.61±1.59	<0.05	<0.05
Maximum Rate of Expiration (MEF_{25}), %	93.1±4.81	71.45±2.91	73.18±2.87	76.6±2.4	<0.05	<0.05
Maximum Rate of Expiration (MEF_{50}), %	90.5±4.11	73.04±2.23	76.68±2.05	80.59±2.1	<0.05	<0.05
Maximum Rate of Expiration (MEF_{75}), %	89.9±3.98	66.38±2.16	70.42±2.28	76.84±2.03	<0.05	<0.05
FEV_1/FVC, %	84.2±2.47	80.69±2.34	83±2.15	88.19±1.77	<0.05	<0.05

Notes:

p1 – reliability of differences of parameters between subgroups of studied and practically healthy persons;

p2 – significant difference between the indexes before and after the treatment;

p3 – significant difference of indexes between subgroups of the study.

Table 2. The indicators of excretory lung function during treatment of patients with infiltrative tuberculosis depending on the presence of chronic bronchitis, (M±m)

Indexes of spirometry	Groups of the studied persons				p1	p2	p3
	Practically healthy individuals (PHI), n=15	group II, n=22					
		Before treatment	30th day	150th day			
Forced Volume Vital Capacity (FVC) %	101.2±3.25	90.64±0.81	91.45±0.51	92.83±0.41	<0.05	<0.05	<0.05
Forced Expiratory Volume (FEV_1), %	97.3±1.47	82.63±0.9	87.89±0.77	90.6±1.08	<0.05	<0.05	<0.05
Maximum Rate of Expiration (MEF_{25}), %	93.1±4.81	69.95±2.28	75.33±2.43	89.12±1.83	<0.05	<0.05	<0.05
Maximum Rate of Expiration (MEF_{50}), %	90.5±4.11	75.15±2.00	78.62±1.82	88.58±1.55	<0.05	<0.05	<0.05
Maximum Rate of Expiration (MEF_{75}), %	89.9±3.98	65.9±2.12	73.06±2.11	82.46±2.19	<0.05	<0.05	<0.05
FEV_1/FVC, %	84.2±2.47	80.27±1.89	85.37±1.54	91.6±0.95	<0.05	<0.05	<0.05

Notes:

p1 – reliability of differences of parameters between subgroups of studied and practically healthy individuals;

p2 – significant difference between the indexes before and after the treatment;

p3 – significant difference of indexes between subgroups of the study.

mechanisms of liquidation of combination of specific and nonspecific inflammation in the broncho-pulmonary system, and thus – the reduction of the basis for secondary bronchial obstruction.

During 30 days of the standard treatment regimen of patients of group I, the concentration of IL-6 had a small positive dynamics (Table 3). During the treatment at the 30^{th} day in the group I, we have seen a slight positive impact of the standard treatment regimen for this index, level of which has decreased at 1.12 times and amounted (184.12±14.39) pg/ml. The best indexes we have marked at the 150^{th} day of the standard regimen of group I, where the reduction of IL-6 in BAC was established, namely a reduction at 1.72 times ($p2<0.05$) as compared to the indexes before treatment. However, the level of IL-6, despite the described positive dynamics, in the study group I remained at 2.62 times higher than the indexes of the group of PHI ($p1<0.05$).

It should be noted that the combined therapy with the inclusion of glucosaminilemuramylpentapeptide was more effective for the normalization of IL-6. According to the 30^{th} day of the treatment, we observed (132.85±10.53) pg/ml ($p2<0.05$), which was better at 27.85% for the corresponding period in the group I ($p3<0.05$) and at 34.44% better as compared to the indexes before treatment ($p2<0.05$).

Table 3. Change of the level of IL-6 (pg/ml) in bronchoalveolar content in the course of treatment of patients with infiltrative tuberculosis combined with chronic bronchitis, (M±m)

Indexes	PHI, n=15	group I, n=11			group II, n=16			p1	p2	p3
		Before treatment	30 days of treatment	150 days of treatment	Before treatment	30 days of treatment	150 days of treatment			
IL-6 (pg/ml)	45.52 ±4.14	205.48 ±11.6	184.12 ±14.39	119.27 ±6.96	202.64 ±15.31	132.85 ±10.53	68.79 ±8.68	<0.05	<0.05	<0.05

Notes:

p1 – reliability of differences of parameters between subgroups of studied and practically healthy persons;

p2 – significant difference between the indexes before and after the treatment;

p3 – significant difference of indexes between subgroups of the study.

At the 150th day of treatment in group II of study, we have noted positive dynamics of IL-6 level in BAC, which significantly decreased (p2<0.05). The achieved effect at 42.32% exceeded the same index in study group I. However, this index remained 1.5 times higher than the values of the PHI group (p1<0.05).

Prospects for further research. It is advisable to develop some effective ways to influence the pathogenic level with comorbidity to improve the effectiveness of treatment and quality of patients' life with infiltrative tuberculosis combined with chronic bronchitis.

3. Conclusions

1. The additional inclusion of glucosaminilemuramylpentapeptide into the complex therapy of patients with infiltrative tuberculosis combined with chronic bronchitis in acute phase manifests itself by positively aimed dynamic changes of the bronchial mucous membrane according to endoscopy data, and the best result was observed in patients who in the complex treatment took two courses: 16 patients – intramuscularly at a dose of 2 mg, 5 injections with an interval of 5 days; in 3 months (12 patients) – the second course lasting 20 days, 1 tablet at a dose of 2 mg 2 times a day of this immune modulator.

2. Inclusion of glucosaminilemuramylpentapeptide into the complex treatment of patients with infiltrative tuberculosis and chronic bronchitis in acute phase by two courses provides stabilization of the respiratory function and normalization of parameters of IL-6 in bronchoalveolar content.

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